REMARKS

1. Non-Art Issues

In 112/2 rejection (OA §4), the Examiner rejects claims 40 and 61 (and by virtue of dependency 60-79 and 85-90) because they recite "disease associated". The Examiner explains, "it is unclear how to determine and what are the criterias [sic] for which cell [sic] are associated with disease and which are not associated with disease".

However, Applicants believe that, when read in the light of the specification, it is clear that the claim should be parsed so that "disease associated" modifies "antigen", not "T cells". The Examiner seems to recognize that this was the intent of the disclosure.

The Examiner comments, "if Applicant means a [sic] cytotoxic T cells that were activated by a disease-specific antigen he should clearly stated [sic] this for clarity and consistence [sic] with the disclose [sic] of the specification".

We are not sure what further distinction, if any, the Examiner is drawing between "disease-associated" and "disease-specific". While there is basis for "disease-specific" at page 21, lines 24-26, there is far more discussion of "disease-associated", see, e.g., page 10, lines 29-35, and hence we think retention of that term is desirable. Claims 40 and 61, and withdrawn claim 80, have been amended to recite that the T cells are activated by a "disease associated antigen". Nonetheless, in the interest of compact prosecution, we have presented new claim 92, reciting "disease-specific".

2. Art-Related Issues

Claims 40, 60-79 and 85-90 stand rejected under 35 USC 102(e) as allegedly anticipated by Riddell, USP 5,827,642 or Flyer, USP 6,316,257 (OA page 2). The same claims also stand rejected as allegedly anticipated under 102(b) by Habermann, USP

5,188,959 (OA page 4, note the duplicate section "6"). These rejections are respectfully traversed.

The Examiner concedes that the '642 patent does not explicitly teach that its cytotoxic T cells have exceeded a lifespan of 40 (claims 40, 61) 50 (claim 89), 60 (claims 85 and 90), 100 (claim 86), 150 (claim 87) or 200 (claim 88) PD (population doublings), but contends that the '642 teaches the "same" culturing methods and conditions as those taught herein, and hence that such lifespans are inherent properties of the prior art T cell cultures.

All normal somatic cells, including cultured T cells, undergo replicative senescence, whereby the cells are capable of only a finite number (known as the Hayflick limit) of cell divisions in tissue culture before reaching an irreversible state of growth arrest. Replicative senescence is a consequence of cell division, rather than chronological age. With appropriate feeding (feeder cells) such senescent cultures can be maintained in a viable, non-proliferative state for many months. See Effros (1997) (ref. BR), at page 451, col. 2.

The key role of telomerase activity in determining replicative senescence was demonstrated by Hooijberg et al, J Immunology., 165:4239-4254 (2000) where ectopic recombinant expression of telomerase reverse transcripitase (TERT) in a CD8+ T cell clone dramatically extended the life span of the clone. Loss of telomerase activity resulting in the progressive loss of telomeric DNA is widely recognized to be a primary cause of senescence in somatic cells (including T-cells).

The Hayflick limit was first proposed in 1961, and since that time the scientific community has consistently upheld the view that isolated T-cells, grown in vitro (even in the presence of cytokines), are not capable of replicating beyond approximately 23 PDs, without genetic manipulation. See, for example, Effros (1997), supra; Kaltoft, "Cytokine' Driven

Immortalization of In Vitro Activated Human T-lymphocytes", Exp. Clin. Immunogenet. 15:84-89 (1998); Roth, et al., Blood, 102(3):849-57 (2003).[1]

Scientists have sought to overcome replicative senescence in order to provide a sufficient number of cells for clinical uses, such as adoptive transfer therapy. Attempts to up-regulate telomerase activity in T-cells in vitro by stimulation with a specific antigen, mitogens, or anti-CD3/anti-CD 28 antibodies are only temporarily effective, and repeated cycles of stimulation lead to a progressive lowering of the telomerase peak and do not prevent or delay the onset of replicative senescence. See Migliaccio et al, J Immunology, 165:4978-4984 (2000).

The three prior art documents cited by the examiner provide experimental documentation for the in vitro expansion of T-cells, but in no case do these documents demonstrate expansion to the claimed 40 PD.

The present invention is thus the first disclosure of immortalized (proliferation beyond Hayflick limit) cytotoxic T-cells which does not involve recombinant expression of TERT. The immortal cytotoxic T-cell lines of the present invention have exceeded a life span of at least 40 PD because the cells have been activated by a disease-associated antigen, and have thereby escaped replicative senescence. The antigen-activated cells are characterized by cytokine-dependent immortalization, allowing their growth in the presence of just two cytokines [p.9, l.33-34; p.13, l.10-12]. The reason for the immortality of the cell line

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[1] The figure of 23 PD is given in the specification, at P1, L36, and in the Kaltoft Declaration (there, 23± 7 PDs). Effros, page 451, col. 1, in discussing CD8+ (cytotoxic) T cells, says that these senesced at 23 PDs. Effros gives higher senescence limits for CD4+ cells and for bulk cultures of uncloned T cells. However, the present claims are directed to cytotoxic T cells. Hooijberg page 4241 col. 1 suggests a limit of 20-30 PD for CD8+ cells.

of the invention lies in their high telomerase activity (as illustrated by the allostimulated immortal cell lines in Example 1), which is not seen in finite (non-immortal) cell lines. Thus the present invention, by demonstrating the maintenance of telomerase activity in immortal cytotoxic T-cell lines, provides convincing evidence that the cell lines have escaped the Hayflick limit.

The examiner argues that the T-cells of the prior art are produced by methods that are "the same" as those of the present invention, in that both methods include two cytokine growth factors to drive cell expansion. Hence, the Examiner infers that they, too, must have escaped the Hayflick limit. However, the methods are different. Specifically, those employed for propagation of T-cells in the three cited prior art documents all include PBMCs as feeder cells. Experimental observations published by the applicant demonstrate that natural killer (NK) cells, which are known to be present in PBMC feeder cells, effectively eliminate the immortal T-cell lines of the invention. Hence, the skilled person following the protocols provided by the cited prior art would not have arrived at the present claimed invention because feeder cells include natural killer cells, which eliminate the activated cells which might otherwise be proliferated past the Hayflick limit. The evidence for this analysis is reviewed in more detail below.

First, as to the use of PBMCs by the prior art, we direct the Examiner's attention to the following:

Riddell, col. 6, lines 52-57 teaches "adding to the culture medium a disproportionately large number of non-dividing peripheral blood mononuclear cells (PBMC) as feeder cells, such that the resulting population of cells contains at least about 40 PBMC feeder cells for each T lymphocyte in the initial population to be expanded. See also Riddell Example 3.

Flyer discusses Riddell's protocol at cols. 6-7, and seeks

to rapidly expand T lymphocytes in vitro without using a "vast excess" of PBMC or EBv-LCL feeder cells. While a wish is expressed that one could "replace them entirely" (col. 7, lines 33-35). Flyer aptly describes his method as "low-PBMC-REM" (col. 8, line 15). EBV-LCLs are omitted, but PBMCs are still used (col. 13, lines 20-34). Flyer teaches use of PBMCs in a ratio of two PBMCs for every resting T cell (col. 18, lines 25-26), and warns that they are necessary to prevent a 90% loss in viability.

Habermann teaches use of irradiated PBMC to "reactivate" T cells (col. 23, lines 63-65) as well as their use as feeders (col. 25, lines 7-12).

In contrast, the present specification usually teaches against use of feeder cells. See, e.g., page 64, lines 18-19; page 67, lines 16-19; and see new claim 91.

Secondly, as to the presence of natural killer cells among the PBMCs, this is admitted by Flyer at col. 23, lines 12-13. See also Habermann, col. 25, lines 54-58. Janeway, Jr. and Travers, Immunobiology: The Immune System in Health and Disease (copy enclosed) says in section 8-19 that NK cells "make up a small fraction of peripheral blood lymphoid cells".

Thirdly, it is known that NK cells can kill off continuous T cell lines.

Continuous T-cell lines established from patients with atopic dermatitis and activated by the inflammatory process at the site of the disease, (thought to be genetically aberrant) are eliminated by peripheral blood cells in vitro during cultivation, as described on p. 47, line 14-17 in Kaltoft et al Arch. Dermatol Res 287: 42-47 (1994). Further the authors (p. 71, para. 3 in Kaltoft et al, Cancer Genet Cytogenet: 85: 68-71 (1995) show that "peripheral blood mononuclear cells (PBMCs) [sic] derived from patients with benign inflammatory skin diseases do not give rise to continuous T-cell lines, in contrast to T-cells established from a skin biopsy at the site of disease". The authors point to

the NK cells, present in PBMC, as the cause of the elimination of these continuous T-cell lines. "It should be noted that natural killer cells are not present in the skin and that autologous lymphokine-activated killer cells can eliminate the skin-derived cytokine-dependent continuous T-cell lines established from patients with atopic dermatitis". See also the enclosed Kaltoft Declaration.

It is true that the production of immortalised T-cell lines, capable of exceeding 40 PDs, requires the presence of cytokines in the *in vitro* growth medium. However, since native non-activated T-cells, grown in these media, do not show immortality, it must be concluded that the presence of two or more cytokines in the growth media is not sufficient to induce immortalisation of T cells. Rather an initial activation step (either *in vivo* by antigens associated with a disease, preferably a chronic inflammation disease, or *in vitro* by alloactivation of T cells by a continuous T-cell line of the invention) must provide the necessary trigger to induce immortalisation, where the subsequent T-cell growth is cytokine dependent. The disease associated antigen activated T cells, whether activated in vivo or in vitro, are cultivated in media in the absence of PBMCs feeder cells and hence they are not repeatedly exposed to active NK killer cells. In the prior art, those NK cells are present in the irradiated PBMCs used in every amplification cycle.

The discovery that the immortal T cell lines of the invention, retain the ability to express telomerase at levels equivalent to malignant cell lines (see example 1 and Kaltoft 1998, Exp Clin Immunogenet 15:84-89.), provides at least one of the reasons as to why these cells are able to grow continuously, in contrast to native T cells that have not been exposed to disease associated antigen activated T cells. As taught in the article of Röth et al. Blood 102(3) 849-857 (2003), telomerase levels control the lifespan of human T lymphocytes. As explained

by Röth, (see p. 853, para 4), telomerase expression can be activated in native (wild-type) T-cells, but after 36PDs this response is totally lost, and this loss of telomerase (TERT) espression can account for the limited lifespan of native T-cells cultivated in the presence of PBMC feeder cells in their experiments. The authors further confirm that expression of a transgene encoding TERT can elevate telomerase activity to a level sufficient to extend the lifespan of T-cells to at least 60PDs, when these cells are grown with repeated feeder cells and antigen activation.

Besides providing NK cells, which ultimately halt the expansion of the antigen-activated T cells of interest, PBMC feeder cells have other disadvantages, which include those set forth by Flyer at col. 7, lines 22-33. Despite his best efforts in 1996, Flyer was only able to reduce the dependence of REM on PBMC feeder cells, not eliminate it. Thus, in Flyer Ex. 4, $5x10^4$ CTL were co-cultivated with at least $6.12x10^6$ irradiated PBMC (col. 28, lines 35-37).

The present inventors were the first to entirely eliminate the use of feeder PBMCs in the expansion of antigen-activated, cytotoxic T cells. Moreover, they demonstrated proliferation of the CD4+ T cell line Gut_R-2 beyond <u>250</u> PD, with a PD time of about 36 hours (Spec., P55, lines 13-15). See also spec., page 49, lines 32-36, and page 56, lines 9-13.[2]

Since the present inventors did not employ PBMC feeder cells, their protocol was not the "same" as that of the prior art

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[2] We also wish to point out that even if they had that the claimed expected lifespans, certain claims require that the cells have in fact <u>reached</u> the recited PD (claim 40 for 40 PD; claim 89 for 50 PD; claim 90 for 60 PD) and, absent a showing that such event occurred prior to the filing date, those claims would not be anticipated. The factual inquiry is different when a claim recites "have exceeded a lifespan" than when it merely calls for a particular "expected lifespan".

references, and hence it is improper to infer from the present results that the prior methods of Riddell, Flyer and Habermann were inherently capable of breaking the Hayflick limit and reaching the claimed PD levels.

Not only does this mean that the prior art methods do not anticipate claims 40, or 85-94, it also means that they cannot render the claimed product as obvious. See In re Irani, 427 F.2d 806, 807, 809, 166 USPQ 24 (CCPA 1970) (no obvious process of making claimed crystalline anhydrous ATMP, so product is nonobvious); In re Grose, 592 F.2d 1161, 1168, 201 USPQ 57 (CCPA 1979) (zeolite nonobvious); Emory University v. Glaxo Wellcome Inc., 44 USPQ2d 1407 (N.D. Ga. 1997) ((-)enantiomer of BCH-189 patentable since no obvious method of separating the racemic mixture).

3. Procedural Issues

3.1. It is evident that Examiner Saunders read and considered the IDS filed on May 3, 2001, because references AA, AB, AE and AU were cited in his June 13, 2003 action. However, the action did not enclose an initialed PTO-1449, and we request that this oversight be remedied. Also, we notice that the PAIR file history makes no reference to any filing on May 3, 2001, and hence PAIR needs to be corrected.

3.2. A second IDS was filed on October 22, 2001, enclosing a single reference (Gillis, et al., 1978; ref. DA). This PTO-1449 should also be initialed and returned. Also, we notice that PAIR refers to a preliminary amendment having been filed on October 22, 2001, but does not refer to the IDS. It is possible that these were mistakenly treated as a single filing.

3.3. Claims 80-83 should be rejoined pursuant to MPEP 821.04.

Respectfully submitted,

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Citations of Record
-Effros (1997) (ref. BR, of record)
-Kaltoft (1995) (ref. AU, of record)

Enclosures
-Migliaccio (2000) (copy enclosed)
-Kaltoft (1998) (preprint was ref. BJ, of record)
-Roth (2003) (copy enclosed)
-Janeway, Jr. (copy enclosed)
-Hooijberg et al. (2000) (copy enclosed)
-Kaltoft Declaration

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